Identification of UDP Glycosyltransferase 3A1 as a UDP *N*-Acetylglucosaminyltransferase*

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The UDP glycosyltransferases (UGT) attach sugar residues to small lipophilic chemicals to alter their biological properties and enhance elimination. Of the four families present in mammals, two families, UGT1 and UGT2, use UDP glucuronic acid to glucuronidate bilirubin, steroids, bile acids, drugs, and many other endogenous chemicals and xenobiotics. UGT8, in contrast, uses UDP galactose to galactosidate ceramide, an important step in the synthesis of glycosphingolipids and cerebrosides. The function of the fourth family, UGT3, is unknown. Here we report the cloning, expression, and functional characterization of UGT3A1. This enzyme catalyzes the transfer of *N*-acetylglucosamine from UDP *N*-acetylglucosamine to ursodeoxycholic acid (3α, 7β-dihydroxy-5β-cholanoic acid). The enzyme uses ursodeoxycholic acid and UDP *N*-acetylglucosamine in preference to other primary and secondary bile acids, and other UDP sugars such as UDP glucose, UDP glucuronic acid, UDP galactose, and UDP xylose. In addition to ursodeoxycholic acid, UGT3A1 has activity toward 17α-estradiol, 17β-estradiol, and the prototypic substrates of the UGT1 and UGT2 forms, 4-nitrophenol and 1-naphthol. A polymorphic UGT3A1 variant containing a C121G substitution was catalytically inactive. UGT3A1 is found in the liver and kidney, and to a lesser, in the gastrointestinal tract. These data describe the first characterization of a member of the UGT3 family. Its activity and distribution suggest that UGT3A1 may have an important role in the metabolism and elimination of ursodeoxycholic acid in therapies for ameliorating the symptoms of cholestasis or for dissolving gallstones.

The UDP glycosyltransferases (UGT)[2] are a superfamily of enzymes that catalyze the addition of glycosyl residues to small molecular weight lipophilic chemicals (1). This process of glycosylation increases the water solubility of the acceptor substrate and alters its stability and biological reactivity (2–5). The glycosyl donor (co-substrate) is usually a UDP hexose, and during the reaction, the α-bond between UDP and the hexose moiety is converted into a β-bond between the acceptor and the sugar to form a β-D-glycoside. The glycosyl acceptors comprise a structurally diverse array of chemicals and include steroid hormones, bile acids, biogenic amines, plant and bacterial metabolites, carcinogens, and many therapeutic drugs (6). Currently, 80 families containing over 850 UDP glycosyltransferases with diverse substrate specificities have been identified in animals, plants, and microorganisms.[3]

Humans have four UGT families, UGT1, UGT2 (divided into subfamilies 2A and 2B), UGT3, and UGT8 (6). The UGT1 enzymes are encoded by a complex arrangement of nine exons 1A and a shared set of exons 2–5 on chromosome 2q37 (7). Differential promoter usage and splicing produces mature mRNAs that are translated into nine functional UGT1A enzymes, each of which has a unique N-terminal domain encoded by an exon 1A and an identical C-terminal domain encoded by exons 2–5. The UGT1 enzymes use UDP glucuronic acid as sugar donor to glucuronidate bilirubin (UGT1A1), estrogens, bile acids (UGT1A3), tertiary amines (UGT1A4), and numerous other drugs and xenobiotics including carcinogens and bioflavones (3, 8, 10). The UGT2 family contains three members of the UGT2A subfamily and seven members of the UGT2B subfamily. With the exception of UGT2A1 and UGT2A2, which have identical C-terminal domains encoded by a shared set of five exons, all members of the UGT2 family are encoded by separate genes of six exons arrayed along chromosome 4q13 (6). The UGT2 proteins also use UDP glucuronic acid as sugar donor to facilitate the elimination of androgens and many xenobiotics and waste products of metabolism. Although the UGT1 and UGT2 family members prefer UDP glucuronic acid as sugar donor, there are examples where other UDP sugars are used. These include the use of UDP glucose by UGT2B7 and UGT1A1 and the use of UDP xylose by UGT1A1 (11–13). However, their activities with these alternate UDP sugars were always much less than that with UDP glucuronic acid. There is no evidence that UGT1 and UGT2 forms use UDP galactose or UDP *N*-acetylglucosamine.

In contrast to the UGT1 and UGT2 families, which contain many members and which are primarily involved in xenobiotic metabolism, the UGT8 family contains only one member, UGT8A1, which has a biosynthetic role in the nervous system (6). UGT8A1 is encoded by a gene of five exons on chromosome 4q26 and catalyzes the transfer of galactose from UDP galactose to ceramide, an important step in the biosynthesis of the glycosphingolipids, cerebrosides, and sulfatides of the myelin sheath of nerve cells (14).

The existence of the UGT3 family was first noted in 2000 after the analysis of databases assembled as part of the Human

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[2] The abbreviations used are: UGT, UDP glycosyltransferase; HEK, human embryonic kidney.

[3] Update by the UGT Nomenclature Committee.

Genome Project.[4] This family contains two members, which were named UGT3A1 and UGT3A2 by the UGT Nomenclature Committee and which are encoded by genes of seven exons positioned adjacent to each other on human chromosome 5p13.2 (6). However, in contrast to the extensive studies on the function of the UGT1, UGT2, and UGT8 families, and despite much effort, the catalytic properties of the UGT3 family remain an enigma. In this study, we identify UGT3A1 as a UDP *N*-acetylglucosaminyltransferase.

EXPERIMENTAL PROCEDURES

cDNA Cloning and Expression—Human kidney and liver RNA (Stratagene) was used as template to synthesize first strand cDNA with the SuperScript™ first strand synthesis system (Invitrogen). The coding region of UGT3A1 mRNA (Gen-Bank™ reference number BC068446) was amplified from this cDNA using the forward primer, 5′-AGTA<u>CTCGAG</u>TGCTT-CTGTGGAAGTGAGC*ATG*GT-3′, and the reverse primer, 5′-AGTA<u>GGATCC</u>*TCA*TGTCTTCTTCACCTTCCTGGC-3′. The forward primer contained an XhoI site for cloning (underlined) and the UGT3A1 initiation codon (in italics). The reverse primer contained a BamHI site for cloning (underlined) and the stop codon (in italics). PCR was performed in a volume of 20 μl with 200 ng of cDNA, 100 ng of the forward and reverse primers, and the DNA polymerase, *Pfu* Turbo (Stratagene). The cycling parameters consisted of one cycle at 95 °C for 1 min and then 34 cycles of 95 °C for 0.75 min, 61 °C for 0.75 min, 72 °C for 4 min followed by a single 10-min cycle at 72 °C. After electrophoresis on a 1% agarose gel, PCR products of the predicted size were excised and purified from the gel using the QIAquick gel extraction kit (Qiagen) and subcloned into the pCR2.1 shuttle vector (Invitrogen) for sequencing. DNA sequencing revealed that the UGT3A1 insert contained a T361G nucleotide change resulting in a C121G substitution. As Cys-121 is conserved in all mammalian UGTs, mutagenesis with the QuikChange mutagenesis kit (Stratagene) was performed to produce cDNA for the reference protein with a cysteine at position 121. Both UGT3A1 cDNAs encoding the Cys-121 and Gly-121 variants were then cloned into the pEF-IRESpuro6 expression vector, which contains a puromycin resistance gene (15). Expression vectors containing UGT3A1 in either the forward or the reverse direction were transfected into human embryonic kidney (HEK293T) cells, and cell lines stably expressing UGT3A1 proteins were selected with puromycin (2 μg/ml). Expressed UGT3A1 was analyzed by Western blotting and enzyme activity assays.

Production of Antibody—Antibody against UGT3A1 was prepared by using amino acids 57–102 as antigen. This region, which has the least similarity to corresponding regions in other UGTs, was amplified from the UGT3A1 expression vector by PCR with 5′-AGTA<u>GGATCC</u>GC*ATG*CATCAGAGTGGAA-AGTTTTTGA-3′ and 5′-AGTA<u>CTCGAG</u>TCTTCCTTCGA-TATCCAATGCTGTTTCTATGTA-3′ as the forward and reverse primers, respectively. The forward primer contained a BamHI site (underlined) and an initiation codon (in italics). The

reverse primer contained an XhoI site (underlined) and a factor Xa cleavage site. After digestion with BamHI and XhoI, the PCR product was cloned between the BamHI and XhoI sites of the pET23a bacterial expression vector (Novagen). *Escherichia coli* (BL21-DE3) was transformed with the vector and expressed UGT3A1 antigen, which now contains a His$_6$ C-terminal tag encoded by the vector, was purified on a nickel-nitrilotriacetic acid column (Qiagen) and used to prepare antibody in rabbits. The specificity of the antibody for UGT3A1 and its lack of reactivity to UGT1 and UGT2 proteins were assessed by Western blotting (see Fig. 2).

Western Blotting—HEK293T cells stably expressing UGT3A1 cDNA in the correct and reverse orientations were harvested in 10 mM Tris-HCl buffer, pH 7.6, containing 1 mM EDTA and lysed by three freeze-thaw cycles and aspiration through a 22-gauge needle. Protein concentration was determined by the Bio-Rad protein assay, based on the Bradford method (16), and aliquots of 15 μg of lysate protein were subjected to SDS-polyacrylamide gel electrophoresis as described previously (17). Following electrophoretic transfer to nitrocellulose membranes, UGT3A1 protein was detected with UGT3A1 antibody and a secondary goat anti-rabbit antibody conjugated with peroxidase (Zymed Laboratories Inc.). Immunocomplexes were visualized with the enhanced chemiluminescent kit (Thermo Fisher Scientific).

Quantitative PCR—The levels of UGT3A1 transcripts in a human tissue RNA panel composed of RNA from whole brain, heart, lung, kidney, testis, liver, stomach, duodenum, and colon (Stratagene) were quantified using a Rotor-Gene 300 (Corbett Life Sciences) thermal cycler. The forward and reverse primers specific for UGT3A1 were 5′-CTATGCTTCATCAGAGTGG-AAAGTT-3 and 5′-GCTTAGCAAATAACTACATTGAG-TCC-3′ which correspond to nucleotides 161–185 and 352–378 of UGT3A1, respectively. The cycling parameters consisted of one cycle at 95 °C for 15 min and then 40 cycles of 95 °C for 10 s, 55 °C for 15 s, and 72 °C for 20 s. Transcript copy number was determined using UGT3A1 plasmid as standard. At the end of 40 cycles, the integrity of PCR products was assessed by electrophoresis on a 1.5% agarose gel with 100-bp DNA markers (New England Biolabs) as a reference to estimate molecular size. The DNA was visualized by staining with ethidium bromide.

Enzyme Assays—All glycosidation reactions were performed in a final volume of 100 μl containing 100 mM phosphate buffer, pH 7.5, 4 mM magnesium chloride, enzyme source (100 μg of HEK293T cell lysate), 250 μM aglycone substrate, and 2 mM [C-14]UDP sugar (0.1 μCi/mmol). To maximize detection of product, some assays were performed with 250 μM substrate and 0.1 mM (2 μCi/mmol) or 0.5 mM (0.4 μCi/mmol) [C-14]UDP sugar. The reactions were started with the addition of UDP sugar and incubations were performed at 37 °C for 1 h, and were terminated with the addition of 200 μl of ethanol. After centrifugation to remove denatured protein, aliquots of supernatant were subjected to thin layer chromatography on silica gel plates (Baker Si250F) in chloroform:methanol:water:acetic acid, in the v/v ratio of 65:25: 4:2. Radioactive products were visualized and quantified by exposure to a Phosphor Screen, which was scanned with a Typhoon 9400 scanner (GE Healthcare). Standard curves with known amounts of C-14 UDP-sugar were constructed to quantify product

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[4] Presented in 2001 at the 10th International Workshop on Glucuronidation and the UDP Glucuronosyltransferases, Hyogo, Japan.

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                                                      E1 E2
3A1  M-V-GQ--R--VL-LLVAFLLSG-VLLSEAAKILTISTLGGS YL-LLDRVSQILQEHGH   51
      *  *  *   **  **  *   * **  ***** *      ***  **  *  *   **
1A1  MAVESQGGRPLVLGLLLC-VL-GPVV-SHAG KILLIP-VDGS WLSMLGAIQQ-LQQRGH   55


                        E2 E3
3A1  N-VTM-----LH-QSGKF-L----IPDIKEEE-K-SYQV-I--RWFSPE-D-H-QKR-I     89
        *       *   * *  *    *   *  *   *  *    *  *  *   *  *
1A1  EIVVLAPDASLYIRDGAFYTLKTYPVP-FQREDVKESF-VSLGHNVF--ENDSFLQ-RVI   110


              E3 E4
3A1  KKHFDSYIETALDGRKESEALVKLMEIFGTQCSYLLSRKDIMDSLKNE-NYDLVFV AFD   148
      *     *       * * *   *  *  * ** *  * ** *  *     *    *
1A1  K----TY-KK-I--KKDS-AM--LLS--G--CSHLLHNKELMASLA-ESSFDVMLT PF-   153


3A1  F-CS-FLIAEKL-VKP---FV-AILPTTFGSLDF-G--LPSPLSYVPVFP-SLLTDHMDF   197
      **   **     *  *  ** *    * *        *  **     *      *  *
1A1  LPCSP-IVAQYLSL-PTVFFLHA-LPC---SLEFEATQCPNPFSYVPR-PLSSHSDHMTF   206


3A1  WGRVKNFLMFFSFSRSQW--D-MQSTFDNTI-KEHFPEGSRPV-LSHLLLKAELW-FVNS   251
      * **** *  **  *      * *   * *   *   * *  * ** **  **  *
1A1  LQRVKNMLI--AFSQN-FLCDVVYSPYA-TLASE-FLQ--REVTVQDLLSSASVWLF-RS   258


                                  E4 E5
3A1  DFAFDFARPLLPNTVYIGGL--MEKPIKPVPQDLDNFI-ANFGDAGFVLVAF--GS MLNT  306
      ** *  *  *  ***  **    *    * *    *      *  *  **      * **
1A1  DFVKDYPRPIMPNMVFVGGINCLHQ--NPLSQEFEAYINAS-GEHGIV-V-FSLG MVS-  312
                                              E1 E2


3A1  HQSQEVLKK-M--HNAFAHLPQGVIW--TCQSSHWPRDVHLATN-VKIVDWLPQSDLLAH  360
           * **  *   *   ** *  *          * *   **   **  **** *** *
1A1  -EIPE--KKAMAIADALGKIPQTVLWRYT--GTR-P-S-NLANNTI-LVKWLPQNDLLGH  363


              E2 E3
3A1  PSIRL FVTHGGQNSVMEAIRHGVPMVGLPVNGLQ HGNMVRVVAKNYGVSIR-LNQVTA-D  418
      *   *  **  ***  *  ** * ***** *  **    *    *   **   **   *
1A1  PMTRA FITHAGSHGVYESICNGVPMVMMPLFGLQ MDNAKRMETKGAGVTLNVL-EMTSED  422
      E3 E4


3A1  TL-TLTMKQVIEDKRYKSAVV-AASVILH-SQPLSPAQRL-VGWIDHILQTGGATHLKPY  478
       *   *  *  *          *        *     *       *   **    *
1A1  -LEN-ALKAVINDKSYKENIMRLSS--LHKDRPVEPLD-LAVFWVEFVMRHKGAPHLRPA  477
                          E4 E5


3A1  AFQQPWHEQY--LIDVFV-FLLG--LTLG--TMWLC--G--KLLGVVARWLRGARKVKET  523
      *  *  **  ** ** ***  **    *  *  *   * ** *   *  * *
1A1  AHDLTWY-QYHSL-DV-IGFLLAVVLTVAFITFK CCAYGYRKCLGKKGR-VKKAH KSKTH  533
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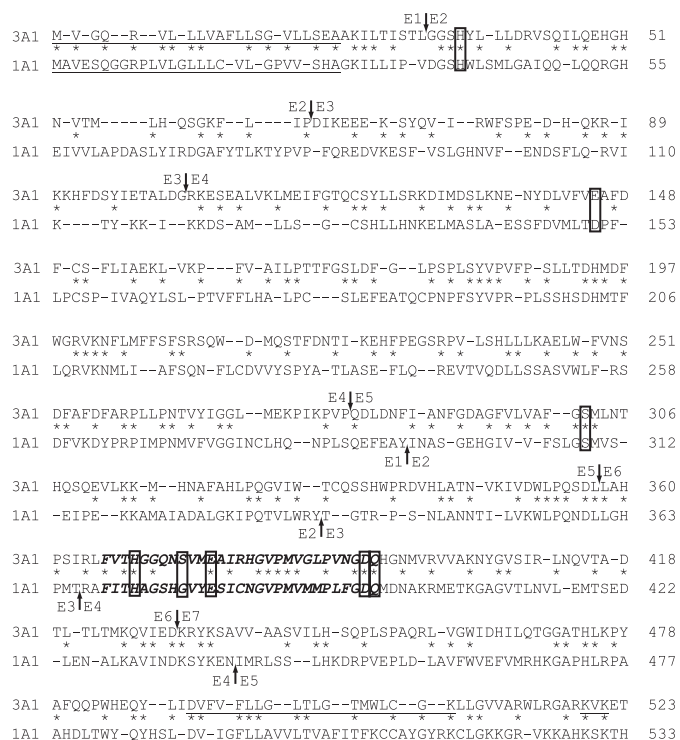
FIGURE 1. Features of the UGT3A1 protein sequence that are conserved in mammalian UGTs. UGT1A1 is aligned below UGT3A1, and the signal peptide, putative transmembrane region, and C-terminal dilysine motif are *underlined*. The signature sequence, which defines the UGT superfamily, is highlighted in *bold italics*. Amino acids considered to be important for catalysis and substrate selection are highlighted in *boxes*. Exon/intron boundaries in UGT3A1 and UGT1A1 are indicated by *arrows*, above and below each sequence, respectively.

formation. Initial experiments established assay conditions to give linear reaction rates with time and protein. Kinetic analyses were performed with ursodeoxycholic acid concentrations ranging from 0 to 250 μM and 2 mM UDP *N*-acetylglucosamine. Kinetic parameters were calculated by fitting experimental data to the Michaelis-Menten equation using EnzFitter (Biosoft). To confirm the presence of *N*-acetylglucosaminide, products formed after incubation with UGT3A1 were extracted with ethyl acetate and digested in citrate buffer (200 mM, pH 5) with Jack Bean *N*-acetyl-glucosaminidase (Sigma) at 25 °C for 16 h. Reductions in the amount of *N*-acetylglucosaminide were revealed by thin layer chromatography as above.

RESULTS

Comparison of UGT3A1 with Other Human UDP Glycosyl-transferases—UGT3A1 is a protein of 523 residues. As with other UGTs (18), it contains a putative signal peptide and a C-terminal hydrophobic region consisting of 17 amino acids between an aspartate and a lysine residue. The signature sequence characteristic of the UGT superfamily and the dilysine motif involved in retention of proteins in the endoplasmic reticulum are also present in the C-terminal half of the protein. A comparison with UGT1A1 as a reference illustrates these conserved features, despite the differences in exon/intron boundaries between the two proteins, which are only 40% identical in sequence (Fig. 1).

As deduced from homology modeling and in some cases mutagenesis experiments, several residues important for sub-
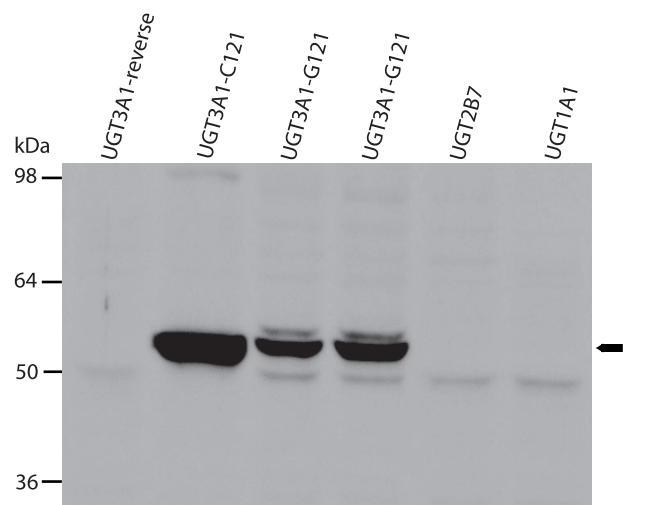

FIGURE 2. Expression of UGT3A1 in HEK293T cells. The synthesis of UGT3A1 in HEK293T cell was examined by Western blotting. The presence or absence of UGT3A1 protein in cells transfected with UGT3A1-Cys-121 in the reverse and forward orientations, two clones of UGT3A1-Gly-121, UGT1A1 and UGT2B7, is denoted by an *arrow*. The molecular mass markers are indicated on the *left* of the figure.

strate binding and catalysis have been identified in the UGT1 and UGT2 enzymes (19–23). Based on UGT1A1 as the reference sequence, the histidine (UGT1A1, His-39), which is thought to deprotonate the acceptor group on the substrate and/or play a role in substrate selection, is conserved in UGT3A1 (His-35). The aspartate residue (UGT1A1, Asp-151), which is thought to stabilize the protonated histidine or be involved directly in proton abstraction, is replaced by a glutamate residue in UGT3A1 (Glu-145). Other residues, purported to be involved in binding substrate and UDP-sugar, are also conserved or subject to conservative replacement. These include the serine (UGT1A1, Ser-309/UGT3A1, Ser-302) and histidine (UGT1A1, His-372/UGT3A1, His-369) residues, which are suggested to form hydrogen bonds with the β-phosphate group of UDP, and the glycine/serine (UGT1A1, Gly-377/UGT3A1, Ser-374), which is thought to form hydrogen bonds with the α-phosphate of UDP. Other residues of the signature sequence that are considered to interact with the hydroxyl groups of ribose are also conserved (UGT1A1, Glu-380, Asp-396, Gln-397/UGT3A1, Glu-377, Asp-393, Gln-394). These features are illustrated in Fig. 1.

Catalytic Properties of UGT3A1—UGT3A1 is synthesized in transfected HEK293T cells as a 53-kDa protein (Fig. 2) and catalyzes the transfer of *N*-acetylglucosamine from UDP-*N*-acetylglucosamine to the bile acid, ursodeoxycholic acid (Fig. 3*A*). The presence of *N*-acetylglucosaminide was confirmed by digestion with *N*-acetylglucosaminidase (data not shown). UGT3A1 displays Michaelis-Menten kinetics toward ursode-oxycholic acid with a K_m of 49 μM and V_{max} of 309 pmol/min/mg of protein (Fig. 3*B*). The *N*-acetylglucosaminidation of the primary bile acids, cholic acid (3α, 7α, 12α-trihydroxy-5β-cholanoic acid) and chenodeoxycholic acid (3α, 7α-dihydroxy-5β-cholanoic acid), and the secondary bile acid, deoxycholic acid (3α, 12α-dihydroxy-5β-cholanoic acid) was not detected, whereas only trace amounts of activity toward other secondary bile acids, including lithocholic acid (3α-hydroxy-5β-cholanoic

FIGURE 4. **UGT3A1-catalyzed glycosidation of ursodeoxycholic acid and 17α-estradiol.** Various UDP sugars were used as co-substrates in the glycosidation of ursodeoxycholic acid and 17α-estradiol by lysates of HEK293T cells transfected with UGT3A1 cDNA in the forward (+) and reverse (−) orientations. An autoradiograph of the TLC plate containing the conjugated products and unreacted UDP sugar and/or its breakdown products from assays with 250 μM substrate and 0.5 mM [C-14]UDP sugars is shown. Equal amounts of radiolabeled UDP sugar (0.4 μCi/mmol) were used in each reaction. *UDP-GlcNAc*, UDP N-acetylglucosamine; *UDP-GlcUA*, UDP glucuronic acid; *UDP-Glc*, UDP glucose; *UDP-Gal*, UDP galactose; *UDP-Xyl*, UDP xylose.

FIGURE 3. **N-Acetylglucosaminidation of ursodeoxycholic acid and other substrates by UGT3A1.** *A*, assays to maximize detection of product, or to determine reaction rates, were performed in the absence of substrate (−) or with 250 μM substrate and 0.5 or 2 mM [C-14]N-acetylglucosamine, respectively, as described under "Experimental Procedures." An autoradiograph of the TLC plate containing the conjugated products and unreacted UDP sugar and/or its breakdown products from assays with 0.5 mM [C-14]N-acetylglucosamine is shown. The reaction rates, determined with 2 mM [C-14]N-acetylglucosamine for each substrate, are given in pmol/min/mg of protein. The limit of detection under these conditions is 5 pmol/min/mg of protein. *, a product was detected, but rates at saturating substrate concentrations could not be accurately calculated. *UDCA*, ursodeoxycholic acid; *CDCA*, chenodeoxycholic acid; *Chol*, cholic acid; *Litho*, lithocholic acid; *Deoxy*, deoxycholic acid; *Hyo*, hyodeoxycholic acid; *17α-Eol*, 17α-estradiol; *17β-Eol*, 17β-estradiol; *4-MU*, 4-methylumbelliferone; *4-NP*, 4-nitrophenol; *1-Nap*, 1-naphthol. *B*, kinetic (*V* versus [*S*]) and Eadie-Hofstee (*V* versus *V*/[*S*]) plots for ursodeoxycholic acid N-acetylglucosaminidation by recombinant UGT3A1. Points are for experimentally determined values, whereas the *solid line* shows the fit of data to the Michaelis-Menten equation.

FIGURE 5. **Detection of UGT3A1 transcripts in a human tissue RNA panel.** UGT3A1 RNA was amplified by reverse transcription-PCR over 40 cycles and detected by electrophoresis in a 1.5% agarose gel, stained with ethidium bromide. The 100-bp DNA markers are shown on the *left* of the figure. The copies of UGT3A1 RNA/20 ng of input tissue RNA were determined during the amplification process by quantitative PCR and are shown below each lane. The lane on the *right* is the PCR product from UGT3A1-expressing HEK293T cells, which served as a reference for product size.

acid) and hyodeoxycholic acid (3α, 6α-dihydroxy-5β-cholanoic acid), were found (Fig. 3*A*). UGT3A1 had negligible activity with other UDP sugars including UDP glucose, UDP glucuronic acid, UDP galactose and UDP xylose (Fig. 4). HEK293T cell lysates contain a compound that is prominently galactosidated, but this compound is not a substrate of UGT3A1 as its galactosidation occurs in HEK293T cell lysates that do not express UGT3A1 (Fig. 4). In addition to ursodeoxycholic acid, UGT3A1 N-acetylglucosaminidates 17α-estradiol, 17β-estradiol, and the prototypic substrates of the UGT1 and UGT2 forms, 4-nitrophenol and 1-naphthol (Fig. 3*A*). Slight activity toward 4-methylumbelliferone was also observed.

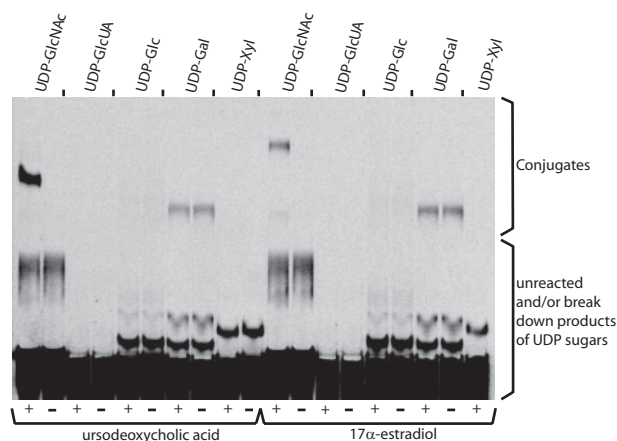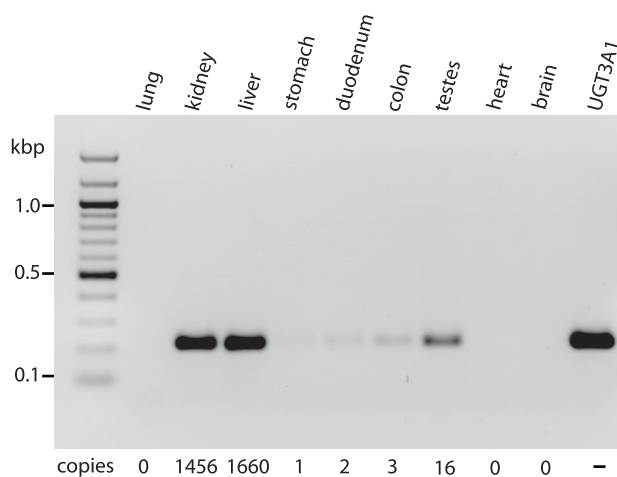During the cloning of UGT3A1, two variants were isolated. These were the Cys-121 variant, whose catalytic properties are described above, and the Gly-121 variant. As the UGT3A1-Gly-121 variant is found to a significant extent in the human population,[5] it was also expressed in HEK293T cells. Two clones that expressed UGT3A1-Gly-121 protein (Fig. 2) were devoid of catalytic activity, even when assayed with ursodeoxycholic acid and 17α-estradiol as substrates, under conditions to maximize detection of product (data not shown).

Distribution of UGT3A1—As demonstrated by quantitative PCR, transcripts encoding UGT3A1 were detected in human kidney and liver (Fig. 5). Small amounts of transcript could also be detected in stomach, duodenum, colon, and testes but were

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[5] Data from the Reference SNP Cluster Report: rs3756669.

undetectable in heart, lung, and whole brain (Fig. 5). As with HEK293T cells, UGT3A1 transcripts were not present in other cultured cell lines such as HepG2, Caco-2, MCF7, and LNCaP (data not shown). The presence of UGT3A1 in the liver and kidney was also confirmed by Western blotting (data not shown).

DISCUSSION

Although *N*-acetylglucosaminides of small molecular weight compounds including ursodeoxycholic acid have been reported previously, the enzyme involved was not identified (24, 25). In this work, we identify UGT3A1 as this enzyme and show that it is mostly expressed in the liver and kidney. Ursodeoxycholic acid is a low abundant secondary bile acid formed by the bacterial epimerization of the 7α-hydroxy group of chenodeoxycholic acid and appears to be of little physiological significance in the healthy adult. However, ursodeoxycholic acid is the only bile acid currently recommended for treating liver dysfunction in patients with cholestatic liver diseases and for dissolving gallstones (26, 27). It appears to be hepatoprotective, as it reverses hydrophobic bile acid hepatotoxicity by activating pregnane-X-receptor and inducing CYP3A4 (a bile acid-metabolizing enzyme) in primary human hepatocytes (28). Dosage with ursodeoxycholic acid leads to profound changes in the composition of bile acids in bile and urine. For example, after daily treatment (10–15 mg/kg) for 2–3 weeks, 50% of the total bile acids in the serum, urine, and bile of gallstone patients consists of ursodeoxycholic acid when compared with 3–4% in untreated subjects (29). Under these conditions of increased ursodeoxycholic acid load, the major metabolite in the urine appears to be the *N*-acetylglucosaminide, with the sugar attached to the 7β-hydroxyl group (25, 30). Hence UGT3A1, which catalyzes this reaction, is likely to be of major significance in these pathophysiological states of bile acid overload.

UGT3A1 displays Michaelis-Menten kinetics with ursodeoxycholic acid (K_m 49 μM, V_{max} 0.31 nmol/min·mg of protein) and appears to prefer this bile acid, as other bile acids are either poorly *N*-acetylglucosaminidated or not *N*-acetylglucosaminidated. This is in agreement with previous work on unidentified hepatic and renal *N*-acetylglucosaminyltransferase, which were specific for ursodeoxycholic acid and had little activity toward the primary bile acids, chenodeoxycholic acid and cholic acid, and the secondary bile acids, lithocholic acid and deoxycholic acid (31).

The selectivity of UGT3A1 for ursodeoxycholic acid also reflects the situation *in vivo*. Only glucuronides and glucosides of other bile acids are detected, especially under conditions of impaired bile flow (32, 33). These include the glucuronides of chenodeoxycholic acid, cholic acid, lithocholic acid, deoxycholic acid, and hyodeoxycholic acid. Chenodeoxycholic acid and deoxycholic acid are mainly glucuronidated on their C-24 carboxyl group (34), hyodeoxycholic acid is mainly glucuronidated at the 6α-position, and the other bile acids are glucuronidated on either a hydroxyl or a carboxyl group. UGT1A3 appears to be the major enzyme involved in the glucuronidation of the C-24 carboxyl group (chenodeoxycholic and lithocholic acids) (33, 35). In contrast, UGT2B4 and UGT2B7 preferentially glucuronidate hydroxyl groups on bile acids. The latter

also mediates the glucosidation of hyodeoxycholic acid on the 6α-hydroxy group (11).

In addition to ursodeoxycholic acid, UGT3A1 also *N*-acetylglucosaminidates other compounds including 17α-estradiol. 17α-Estradiol is an endogenous steroid that is synthesized from the aromatization of 17α-testosterone in various tissues including the brain (36). Although a poor ligand for the estrogen receptor and generally regarded as hormonally inactive, 17α-estradiol is as potent as 17β-estradiol in protecting neurons from oxidative stress (37, 38). In this work, we show that of the two β-estradiol stereoisomers, UGT3A1 preferentially conjugates 17α-estradiol. Although the site of UGT3A1-catalyzed *N*-acetylglucosaminidation of 17α-estradiol is unknown, it is likely to be the 17-hydroxyl, as only 17α-estradiol-17-*N*-acetylglucosaminides have been described to date (39). The two β-estradiol stereoisomers are also glucuronidated (35, 40, 41). The 3-hydroxyl groups of both steroids are glucuronidated by UGT1A1, UGT1A3, UGT1A10, and UGT2A1, whereas their 17-hydroxyl is glucuronidated by UGT2B7. UGT2B4 shows specificity for the 17-hydroxyl group of 17α-estradiol (41). The relative importance of glucuronidation and *N*-acetylglucosaminidation in the metabolism of 17-estradiol remains to be clarified.

As well as selectivity for substrate, UGT3A1 also appears to preferentially utilize UDP-*N*-acetylglucosamine as co-substrate, as glycosidated products with other UDP sugars were not detected under the assay conditions used. However, further studies with many substrates are required, as it is possible that UGT3A1 may glycosidate one or more compounds with sugars other than *N*-acetylglucosamine, as was observed with UGT2B7, which can glucuronidate many compounds, but can also selectively glucosidate hyodeoxycholic acid (11).

Using a commercial source of human tissue RNA, we initially cloned a UGT3A1 cDNA, which encoded a glycine at position 121. This corresponded to the only known polymorphism in the UGT3A1 coding region reported to date: a T361G nucleotide change resulting in a C121G substitution.[5] As a cysteine at position 121 is conserved across all mammalian UGT1, UGT2, and UGT8 families, we reasoned that it is likely to be of functional significance. Support for this conjecture was provided by studies with UGT1A6, which showed that substitution of this cysteine (Cys-126 in UGT1A6) with serine or valine partially or completely inactivated the enzyme, respectively (9). In this work, we show that UGT3A1 containing a glycine at position 121 has negligible activity toward ursodeoxycholic acid and 17α-estradiol. The T361G nucleotide polymorphism is present in a homozygous state in about 20% of Asian and Caucasian populations but is absent in African Americans.[5] As this polymorphism yields an inactive protein, the therapeutic use of ursodeoxycholic acid in Asians and Caucasians might be improved by dosage adjustments in patients homozygous for the G-allele. However, further studies are required to determine whether there is a relationship between *N*-acetylglucosaminidation and the therapeutic or toxic effects of ursodeoxycholic acid usage.

The UGT3 family contains two members, UGT3A1 described above, and UGT3A2. As these two enzymes are 78% identical in sequence, it is likely that UGT3A2 is also an

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N-acetylglucosaminyltransferase. However, the substrate and UDP sugar preferences of this UGT remain to be identified.

In summary, we demonstrate that UGT3A1 is a novel UDP *N*-acetylglucosaminyltransferase that appears to function primarily as a drug metabolizing enzyme in human liver and kidney. It is involved in the elimination of ursodeoxycholic acid, 17α-estradiol, and some other xenobiotics. The frequent presence of an inactivating *UGT3A1* allele in the human population may have significant therapeutic and/or toxicological implications.

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